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The interplay of adult and larval time constraints shapes species differences in larval life history

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Abstract. In animals with a complex life cycle, larval life-history plasticity is likely shaped by the interplay of selective factors in both larval and adult stages. A wide interspecific variation in responses to larval time constraints imposed by seasonality has been documented. Few studies have addressed differences among closely related species in the evolutionary trajectories of age and size at metamorphosis and their link with larval growth rate under time constraints. None have considered how species-specific length of the reproductive season affects larval developmental responses to time constraints. We tested in four *Coenagrion* damselfly species whether species with a longer reproductive season, facing a smaller threat of missing out on reproduction, react less to larval time constraints and pre-winter food shortage by accelerating development rate and growth rate, and therefore pay less physiological costs. All species increased development and growth rates under larval time constraints. The magnitude of this increase negatively correlated across species with the length of the reproductive season. Under larval time constraints, only the species exhibiting the longest reproductive season suffered a delayed emergence and a reduced investment in energy storage, yet also showed an increased immune function. Under a longer reproductive season, evolution may favor compensation for larval constraints after metamorphosis. Growth rate was accelerated after pre-winter food shortage to the same extent across species; effects on age and mass at emergence also did not differ among species. Time constraints associated with the length of the reproductive season may predictably contribute to species differences in their response to time constraints imposed in the larval stage. Our study adds empirical proof that the interplay of selective factors in the larval and adult stages may determine life-history plasticity with regard to larval time constraints.

Key words: age and size at transition; compensatory growth; complex life history; Coenagrion *spp.; development rate; growth rate; habitat gradient; metamorphosis; phenotypic plasticity; photoperiod.*

Introduction

Complex life histories with a larval stage specialized in growth and an adult stage specialized in reproduction are ubiquitous (Moran 1994). The age and size at which the transition occurs are tightly associated with fitness (e.g., Altwegg and Reyer 2003, De Block and Stoks 2005). Theory has made huge steps in successfully predicting the responses of age and size to environmental factors (e.g., Abrams et al. 1996, Higginson and Ruxton 2010). Variation in size at transition depends on the relative change of development rate over growth rate (Abrams et al. 1996).

Time constraints imposed by seasonality, reflecting the shortage of time available to successfully finish development and reproduction before conditions deteriorate, are a major driver of larval life-history plasticity. Generally, optimality models predict time constraints to increase development rate, resulting in a smaller size (Rowe and Ludwig 1991, Abrams et al. 1996). Experimental studies have documented such life-history responses (reviewed in Dmitriew [2011]). However, other scenarios with size being kept constant or increasing under time constraints in combination with plastic growth-rate increases have been predicted (Abrams et al. 1996) and demonstrated (e.g., Altwegg 2002, De Block et al. 2008*a*).

Despite wide variation in how species respond to seasonal time constraints, few studies have tried to unravel differences between closely related species in the evolutionary trajectories of age and size at transition and their link with growth rate. While species differences in selection strength on the timing and size at transition may occur across both life stages in animals with a complex life cycle, the focus so far has been on selection differences in the larval stage (Dmitriew 2011). For instance, species occupying short-duration larval habitats such as temporary ponds evolved a faster timing of transition (e.g., Gomez-Mestre and Buchholz 2006, De Block et al. 2008*a*). However, since larval and adult stages are closely tied (Moran 1994, Stoks and Córdoba-

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Aguilar 2012), we can also expect species facing different selection on the timing and size of transition because of constraints in the adult stage (Rowe and Ludwig 1991).

Species differences in the length of the reproductive season are prevalent (Ward and Stanford 1982, Corbet 1999) and may drive species differences in size and the timing of transition to adulthood. In species with a relatively short reproductive season and non-overlapping generations, there should be stronger selection for a synchronized early transition to avoid reaching sexual maturity too late (Nylin et al. 1993, Corbet 1999, Munguia-Rosas et al. 2011). In species with an extended reproductive season, less selection on the timing of the transition is expected because a delay in reproduction should have a limited effect on the availability of mating partners and hence fitness. Thus under larval time constraints, to avoid forfeiting reproduction, species expressing a short reproductive season should increase development rate more than species with a long reproductive season. Species with a relatively short reproduction season are also expected to show a greater larval growth acceleration to keep mass at transition as constant as possible, since they cannot compensate with a later emergence. This also means they would be more likely to pay the costs of faster growth. Those costs can occur both within the larval stage (e.g., increased predation rates due to conspicuous foraging; Brodin and Johansson 2004, Stoks et al. 2005) and within the adult stage (e.g., costs like reduced fat reserves, impaired immune responses, reduced cold resistance; Stoks et al. 2006*a*, Stoks and De Block 2011).

Species differences in perceived time constraints and the associated selection on the timing and size at transition should differentially shape their responses to other stressors in the larval stage. This should be especially true for stressors that slow larval life histories, like transient periods of food shortage (Metcalfe and Monaghan 2001, Metcalfe et al. 2002). To compensate for periods of food deprivation, organisms evolved two strategies: (1) decrease development rate and consequently delay age at transition and (2) increase growth rate ("catch-up growth") when food is abundant again to keep both mass and age at transition constant (Metcalfe and Monaghan 2001). We hypothesize that species with a shorter reproductive season will rely more on the latter strategy and show greater compensatory growth when food is again abundant.

Here, we test the response in larval development rate and growth rate in mediating the timing and size at transition under manipulated seasonal time constraints and transient pre-winter food shortage in the larval stage of four *Coenagrion* damselfly species (Odonata: Zygoptera) that strongly differ in the length of the reproductive season (Fig. 1). To explore the fitness consequences in the adult stage of the observed larval life-history changes, we compare the associated physiological costs of rapid growth induced by seasonal time constraints and compensatory growth induced by a period of starvation on two key physiological components closely linked to fitness in damselflies: fat storage and investment in immune function (Stoks and Córdoba-Aguilar 2012). We made the following a priori predictions: (1) Seasonal time constraints associated with late hatching will result in an increased development rate in all four species. The compensatory increase in development rate will be greater in species with a shorter adult reproductive season, whereas species with a long reproductive season will show less compensation and metamorphosis will be delayed. (2) Similarly, a transient period of food shortage will delay the timing of transition more in species with a longer reproductive season. (3) Because fitness in odonates is closely linked to body mass (Sokolovska et al. 2000), we hypothesize growth rate under seasonal time constraints and compensatory growth rate after a transient period of food shortage to increase in all four species to keep mass at transition as constant as possible. For both, we expect a greater growth increase in species with a shorter reproductive season, as these rely more on an increase in development rate. (4) Rapid growth induced by seasonal time constraints and by transient food shortage will come with physiological costs, namely reduced investment in fat reserves and in immune function. Given their expected higher growth rates, we expect species with a shorter reproductive season to pay higher costs than species with a longer reproductive season.

Methods

Study system

We focus on four European *Coenagrion* species (*C. hastulatum*, *C. mercuriale*, *C. ornatum*, and *C. puella*) that occupy permanent waters and overlap in their distributions across Central Europe (Fig. 1; Askew 2004). Females lay eggs during late spring and summer and larvae hatch ~3 weeks after oviposition. In our region, larvae overwinter once, and emergence occurs the following spring (Sternberg and Buchwald 1999). After emergence, adults undergo a ~1–2 week maturation period where considerable additional mass gain occurs (Anholt et al. 1991). The few data available indicate no distinct species differences in mass gain or in length of the maturation period (Anholt et al. 1991, Sternberg and Buchwald 1999).

All four species emerge in May. There are strong differences in the length of their reproductive seasons (Bellmann 1993, Sternberg and Buchwald 1999, Askew 2004, Hunger et al. 2006), which can be ordered as *C. ornatum* < *C. hastulatum* < *C. mercuriale* < *C. puella* (Fig. 1). *Coenagrion ornatum* and *C. puella* differ by ~2.5 months. Although species can co-occur (Sternberg and Buchwald 1999), factors responsible for the differences in the length of the reproductive season have not been identified. To our knowledge, the reproductive season is rather fixed at the species level. In other parts of Europe, reproductive seasons might differ in date, but

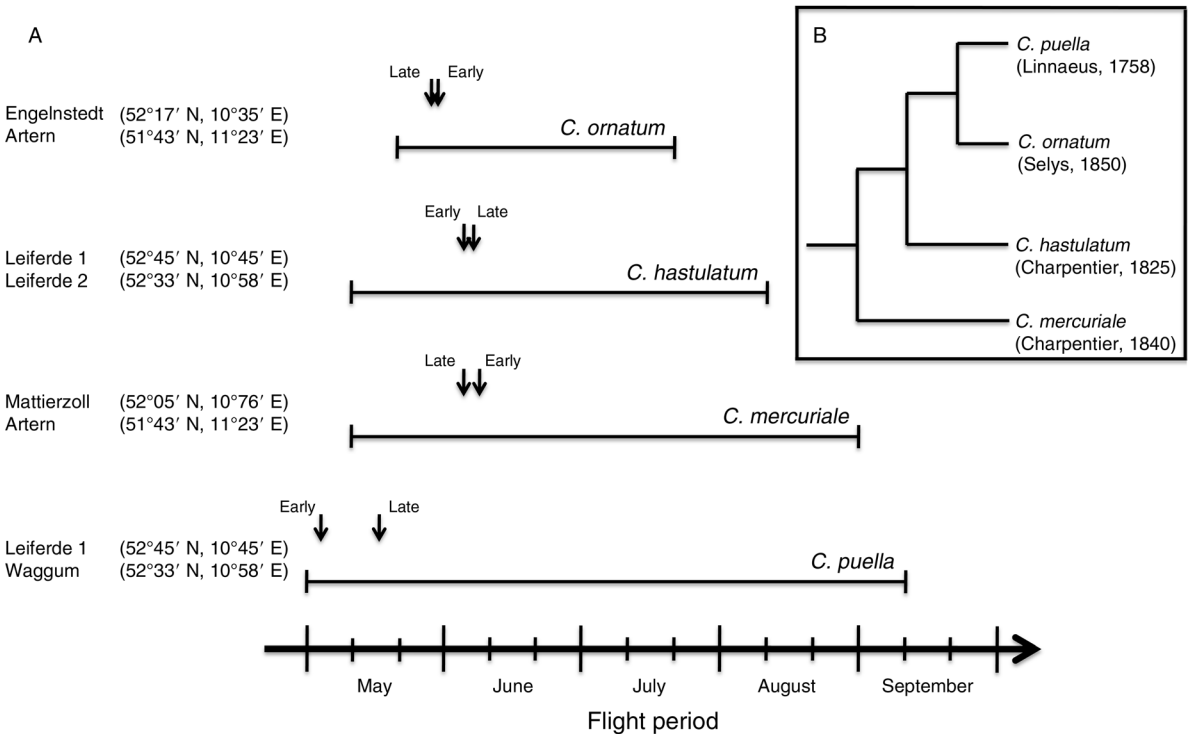

FIG. 1. (A) Length of the reproductive season (flight period) of *Coengrion ornatum*, *C. hastulatum*, *C. mercurial*, and *C. puella* extracted from Bellmann (1993), Sternberg and Buchwald (1999), Askew (2004), and Hunger et al. (2006). Locations in Germany of the source populations are given on the left. Arrows indicate the average emergence day for individuals from early and late photoperiods. Calculation is based on the average day of emergence after 20 March, representing the end of winter in our experiment using a non-intercalary year. (B) Phylogeny for the four *Coenagrion* species, modified from Dumont et al. (2010). Branch length is not incorporated in the tree.

the trend in length of the reproductive season is similar (Askew 2004).

Animal collection and general rearing conditions

Egg clutches of all species were collected during 13–15 June 2005 (Fig. 1) following the method of Mikolajewski et al. (2005). Per species, we collected egg clutches of four different females in each of two populations. Females laid eggs within 48 h of collection. Each egg clutch was divided into four and kept at 21°C in four climate cabinets with corresponding early and late light regimes of the photoperiod treatments. Egg clutches were stored in opaque plastic containers (11 × 18 × 11 cm) filled with 5 cm dechlorinated water. After hatching, larvae were separated in groups of 15 individuals and kept together in opaque plastic containers (11 × 18 × 11 cm) filled with 5 cm water for ~3 weeks (Mikolajewski et al. 2007). We only included larvae that hatched between 6 and 11 July in the experiment. Larvae received *Artemia* nauplii ad libitum twice daily until they were separated.

On 1 August, larvae were put separately into opaque plastic cups (10 cm height, 5 cm diameter) filled to a height of 4 cm with aerated aged tap water. Each cup contained a wooden stick (10 × 0.5 cm) for perching as well as emergence at metamorphosis. Cups were cleaned every other week to minimize algae growth.

Experimental design

We ran a full-factorial rearing experiment where for each species, we crossed two levels of time constraints (early vs. late photoperiod) with two levels of transient pre-winter food shortage (high vs. low food) in the larval stage. Sample sizes for species differed due to egg availability and varied between 28 and 96 animals per treatment combination per species (total of 922 larvae). Sample sizes are given in the Appendix.

We implemented time constraints by manipulating photoperiod following Strobbe and Stoks (2004). Before winter, we applied two photoperiod regimes reflecting situations where larvae of these species hatch early or late in the larval growth season. Directly after egg hatching, one half of the larvae started with a photoperiod matching the natural light regime of 1 June (early photoperiod) and the other half with a photoperiod matching the natural light regime of 1 August (late photoperiod). Light regimes were adjusted every 10 days with the first change occurring when the larvae were separated, thereby following the natural progression in day/night light changes until overwintering. For this, larvae were randomly divided over six temperature cabinets, three for each photoperiod treatment, at 21°C. To prevent any effects from different

cabinets, we randomly changed treatment larvae among cabinets every other week.

When larvae were divided into treatments, we started applying two feeding regimes before winter (Dmitriew and Rowe 2005): half of the larvae received *Artemia* nauplii ad libitum once daily (high food), whereas the other half of the larvae did so only every other day (low food). After winter, to allow compensatory growth, all larvae received *Artemia* nauplii ad libitum twice daily until emergence.

Because *Coenagrion* species undergo a winter diapause during their larval development, we mimicked 14 days of winter conditions when larvae reached the light regime corresponding with 30 October by lowering temperature to 7°C using a daily decrease of 2°C (Strobbe and Stoks 2004). At the end of October, average temperatures in Germany drop below 10°C (German Meteorological Service, Offenbach am Main, Germany) and larval odonate metabolism is largely shut down (Corbet 1999). Overwintering took place in darkness and absence of food. On the first day after the 14 days of winter conditions, the photoperiods in all cabinets were set to the same light regime corresponding with 20 March and temperature was increased by 2°C daily until it reached 21°C (Strobbe and Stoks 2004). This mimics a natural time of the year for larval odonates to start growing again after diapause (Corbet 1999). Again, the natural progression of change in light regime was adjusted every 10 days.

Response variables

We measured wet mass at the start of hibernation, two and five weeks after hibernation, and at emergence. Larvae were gently dried with tissue and weighed to the nearest 0.01 mg. After winter, containers were checked twice daily for newly emerged adults, and adults were sexed. Development time was calculated as the period between egg hatch and emergence as an adult. Each adult was frozen at −80°C for later quantification of fat storage and immune function.

We calculated growth rate based on body mass for two periods: (1) the pre-winter period from hatching to start of winter and (2) the post-winter period covering between two and five weeks after winter. Individual growth rates for the post-winter period were calculated using the formula [ln(mass at end) − ln(mass at start)] divided by the period of time between both measurements (De Block et al. 2008*a*). Since newly hatched larvae cannot be weighed without damage and the initial mass is negligible compared to the mass increase during ontogeny (Johansson and Rowe 1999, De Block et al. 2008*a*), growth rate for the pre-winter period was calculated using the formula ln(mass at start of winter)/(number of days until start of winter). To obtain an estimate of growth rate across the entire larval period, we calculated (3) growth rate based on final dry mass divided by larval period (Johansson and Rowe 1999, Johansson et al. 2001). Using wet mass (1) and (2) or dry mass (3) is not expected to make a difference, given the close association of wet and dry mass across coenagrionid species (c.f. McPeek et al. 2001).

To assess immune function, we scored phenoloxidase (PO) activity, a key enzyme in insect immune function (Siva-Jothy et al. 2005), following the protocol by Rolff et al. (2004). Haemolymph extracts were obtained by perfusing the thorax of an individual with 0.3 mL PBS buffer (pH 7.4; Beckton Dickinson, Erembodegem, Belgium). The cell walls were removed via centrifugation (4°C, 4000 rpm, 15 min). A 100-μL quantity of the supernatant was added to 60 μL L-DOPA (in 10 mmol/L cacodylate buffer) and 40 μL PBS buffer. Enzyme activity was measured spectrophotometrically (plate reader Versamax, Molecular Devices, Sunnyvale, California, USA) as the slope (V_{max}) during the linear phase of the reaction during which PO catalyzes the transition from L-DOPA to dopachrome. Samples were run in duplicate and the mean of each sample was used for analysis. This treatment does not substantially affect any further trait measurements, e.g., fat content (c.f. Rolff et al. 2004, Stoks et al. 2006*b*).

We quantified final dry mass and fat content as good proxies for damselfly fitness (Rolff and Joop 2002). Dry mass was quantified to the nearest 0.01 mg after drying animals for 48 h at 60°C (Mikolajewski et al. 2004). To determine fat mass, dried individuals were placed in a microtube to which we added 1.5 mL dichloromethane. Microtubes were gently shaken for 24 h and individuals were dried again for 48 h at 60°C and weighed to the nearest 0.01 mg (De Block et al. 2008*b*). We calculated fat mass as the difference in dry mass before and after lipid extraction.

Statistical analyses

All analyses were conducted using the open-source program R 3.02 (R Development Core Team 2009). Data were analyzed using linear mixed-effect models with maximum likelihood (package lme4 version 1.0-5; Bates et al. 2013), with populations as a random effect nested within species (Mikolajewski et al. 2010) as well as photoperiod (early/late), food (high/low), and species as independent fixed effects. Data on post-winter growth rate were $\sqrt{x+1}$-transformed to obtain normally distributed residual errors. In all analyses, we used sex as an additive independent fixed effect to control for sex differences in traits (Stoks et al. 2006*b*). Body mass was included as a covariate when analyzing fat content and PO activity (Rolff et al. 2004). Because of different mortality among treatments, the final design was unbalanced. Therefore, we used Type III sum of squares with Wald chi-square statistic to test for fixed effects (package car version 2.0-19; Fox and Weisberg 2013). Data are presented in Figs. 2 and 3 as least-squares means (lsmeans) from the full linear mixed-effect models (package lsmeans 1.10-4; Lenth 2013).

We evaluated interspecific differences in trait plasticity in development and growth rate under time constraints (dependent variable) as a response to

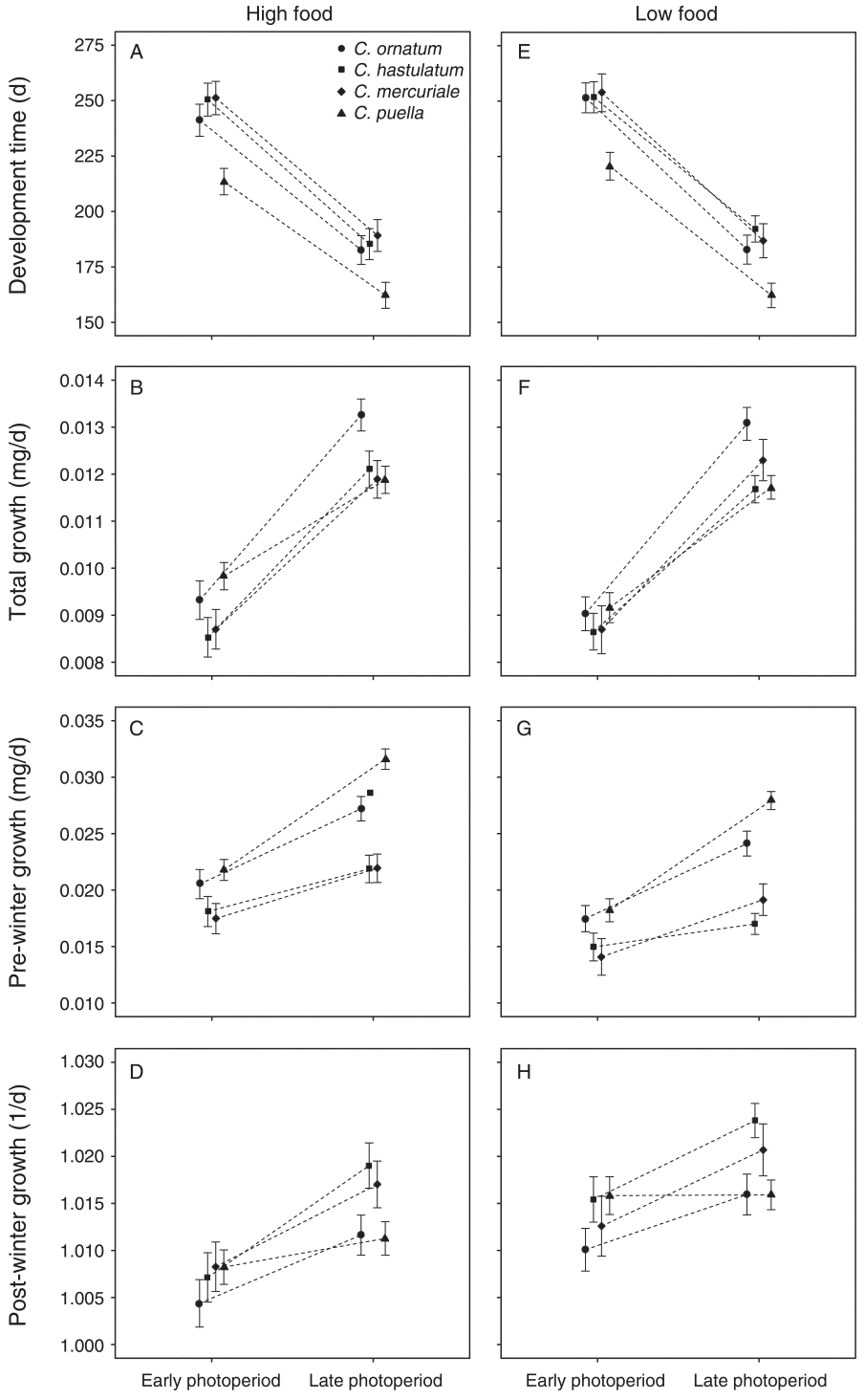

FIG. 2. The effects of photoperiod (early/late photoperiod) and the pre-winter food treatment (high/low food) on (A, E) development time (time from hatching to emergence), (B, F) growth across the total larval period, and (C, G) growth rate in the pre-winter as well as in the (D, H) post-winter larvae period of the four studied *Coenagrion* damselflies. Growth rate in pre- and post-winter larvae is based on wet mass whereas total growth rate is based on dry mass. All rates are given as least-squares means with 95% confidence intervals. Dashed lines are added for better visualization of trait plasticity within species and across treatments.

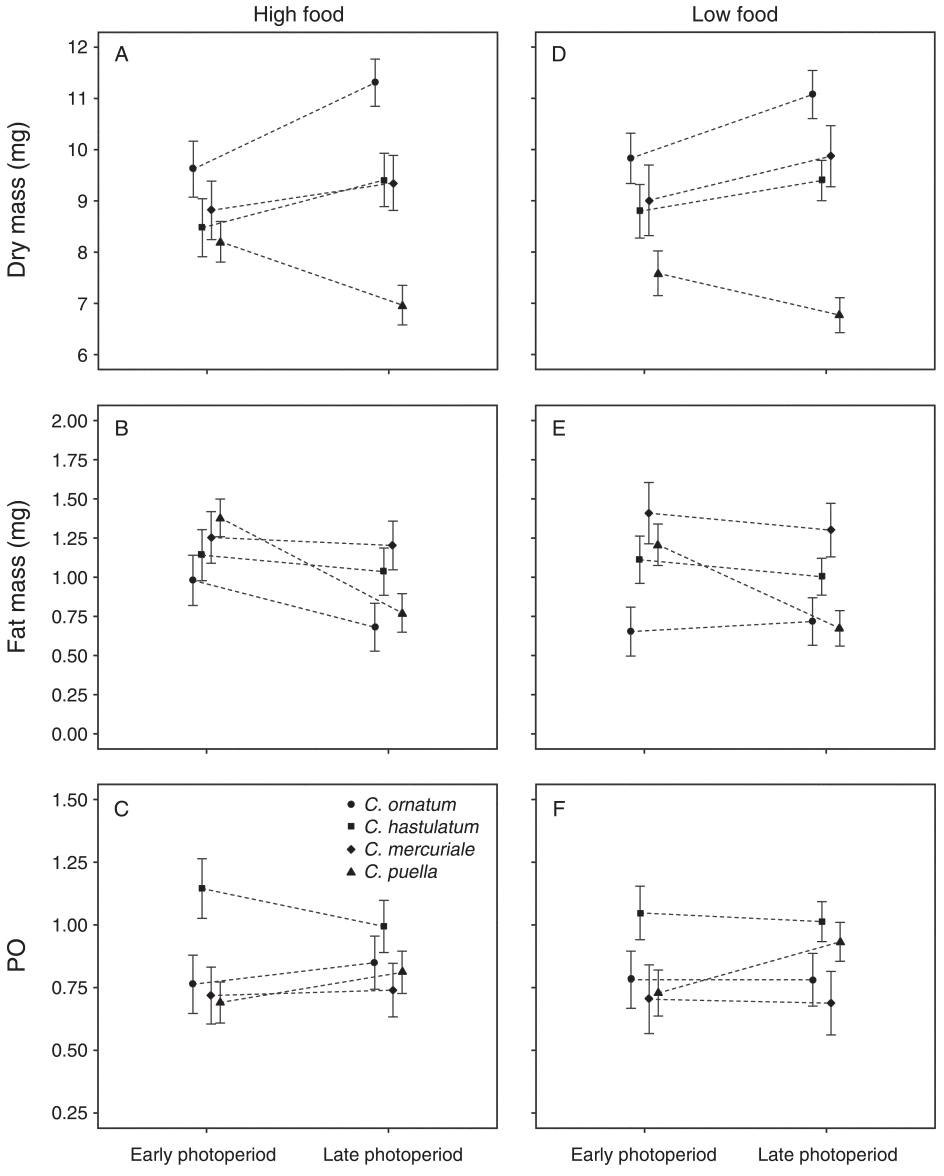

FIG. 3. The effects of photoperiod (early/late photoperiod) and the pre-winter food treatment (high/low food) on (A, D) dry mass, (B, E) fat mass, and (C, F) phenoloxidase (PO) activity at emergence in the four studied *Coenagrion* damselflies. All quantities are given as least-squares means with 95% confidence intervals. Lines are added for better visualization of trait plasticity within species and across treatments.

different lengths of the reproductive season (explanatory variable). To control for relatedness, we applied phylogenetic generalized linear models using the package caper version 0.5.2 (Orme et al. 2013). As a measurement for trait plasticity within species, we calculated average trait difference per species between treatments. This represents a commonly used surrogate for the amount of plasticity (e.g., Via 1993, Money and Agrawal 2008). Length of the reproductive season was taken from Fig. 1. Based on the known *Coenagrion* phylogeny (Dumont et al. 2010; Fig. 1), we analyzed phylogenetically corrected plasticity in development rate and growth rate across the entire development using the extracted least-squares means. Data for high and low food treatments were pooled due to the absence of significant species × food-interactions. We report one-tailed P values due to our strong directional a priori hypotheses. Because of a lack of data on the evolutionary time scale in *Coenagrion* species evolution, branch length was not incorporated in our analysis.

RESULTS

Seasonal time constraints on development rate

Development time (time from hatching to emergence) was considerably shorter in the late compared to the early photoperiod (photoperiod, $\chi^2 = 223.71$, df = 1, $P <$

0.001; Fig. 2A, E), with *C. puella* expressing a shorter development time than the other three species (species, $\chi^2 = 131.32$, df = 3, $P < 0.001$, Figs. 1, 2A, E). Species differed in their response to seasonal time constraints (species × photoperiod, $\chi^2 = 8.65$, df = 3, $P = 0.034$), with *C. puella* accelerating development less under time constraints (~55 days reduction in response to the simulated two month later hatching date) compared to the three other species (~64 days reduction; Fig. 2A, E). Note that percentage of reduction in development time under time constraints was similar in all four species (~25%). This resulted in a pattern of emergence with *C. ornatum*, *C. hastulatum*, and *C. mercuriale* only differing in one to three days comparing early and late photoperiod, whereas in *C. puella* individuals from the late photoperiod emerged 13 days later than individuals from the early photoperiod treatment (Fig. 1).

Across the four *Coenagrion* species, we found a trend for the expected negative phylogenetic covariation between time-constraint-induced plasticity in development rate and length of the reproductive season ($t_2 = -2.14$, $P = 0.083$, $R^2 = 0.545$; slope = −0.112 ± 0.052; all means are shown with standard error). The relatively high R^2 despite the low power of the test, due to using only four species, suggests the pattern to be real.

Pre-winter food shortage on development rate

The period of low food before winter delayed emergence by only ~3 days (food, $\chi^2 = 6.62$, df = 1, $P = 0.01$; Fig. 2A, E), but species did not differ in their response to a period of pre-winter food shortage (species × food, $\chi^2 = 3.01$, df = 3, $P = 0.390$, Fig. 2A, E). There was only a trend toward an interaction between pre-winter food shortage and photoperiod (food × photoperiod, $\chi^2 = 3.49$, df = 1, $P = 0.062$), but no three-way interaction (species × food × photoperiod interaction, $\chi^2 = 4.81$, df = 3, $P = 0.186$; Fig. 2A, E).

Seasonal time constraints and pre-winter food shortage on growth rate

Growth rate across the entire larval period differed among species (species, $\chi^2 = 34.17$, df = 3, $P < 0.001$) and was higher in the late compared to the early photoperiod (photoperiod, $\chi^2 = 221.51$, df = 1, $P < 0.001$; Fig. 2B, F). Species differed in their response to time constraints with *C. puella* (lsmeans difference = 0.0023 ± 0.0001 mg/d) accelerating growth rate less than *C. hastulatum* (0.0033 ± 0.0002 mg/d) and *C. mercuriale* (0.0034 ± 0.0002 mg/d). *Coenagrion ornatum* (0.0040 ± 0.0002 mg/d) increased growth rate more than all other species (species × photoperiod, $\chi^2 = 38.44$, df = 3, $P < 0.001$; Fig. 2B, F).

Pre-winter growth rate differed across species (species, $\chi^2 = 37.74$, df = 3, $P < 0.001$; Fig. 2C, G) and was overall higher in the late than early photoperiod (photoperiod, $\chi^2 = 63.49$, df = 1, $P < 0.001$; Fig. 2C, G). Species differed in their response to time constraints (species × photoperiod, $\chi^2 = 39.29$, df = 3, $P < 0.001$), with *C. puella* (lsmeans difference = 0.0098 ± 0.0005 mg/d) accelerating growth more than *C. ornatum* (0.0067 ± 0.0006 mg/d), followed by *C. mercuriale* (0.0048 ± 0.0007 mg/d) and *C. hastulatum* (0.0029 ± 0.0006 mg/d; Fig. 2C, G). Post-winter growth rate did not differ among species (species, $\chi^2 = 6.93$, df = 3, $P = 0.074$). Species differed in their response to photoperiod (photoperiod, $\chi^2 = 19.38$, df = 1, $P < 0.001$; species × photoperiod, $\chi^2 = 18.06$, df = 3, $P < 0.001$). Whereas *C. hastulatum* (lsmeans difference = 0.0101 ± 0.0012 d^{-1}), *C. mercuriale* (0.0084 ± 0.0014 d^{-1}), and *C. ornatum* (0.0066 ± 0.0012 d^{-1}) increased growth rate strongly under late in comparison to early photoperiod, larval growth rate in *C. puella* (0.0016 ± 0.0009 d^{-1}) did not differ (Fig. 2D, H).

We detected a significant negative phylogenetic covariation between time-constraint-induced plasticity in growth rate and the length of the reproductive season ($t_2 = -4.45$, $P = 0.023$, $R^2 = 0.862$). A shorter reproductive season is accompanied by a greater increase in growth rate as a response to time constraints (slope = $-2.253 \times 10^{-5} \pm 5.065 \times 10^{-6}$).

Pre-winter food shortage on growth rate

There was no effect of the food manipulation in any combination with species and/or photoperiod on growth rate across the entire larval period (all $P > 0.119$; Fig. 2B, F). However, pre-winter larval growth rate was higher in all four species under high food in comparison to low food (food, $\chi^2 = 12.69$, df = 1, $P < 0.001$; Fig. 2C, G). This food effect was similar across species (species × food, $\chi^2 = 0.25$, df = 3, $P = 0.968$; Fig. 2C, G) and was not affected by photoperiod (food × photoperiod, $\chi^2 < 0.01$, df = 1, $P = 0.978$; species × food × photoperiod, $\chi^2 = 1.99$, df = 1, $P = 0.575$; Fig. 2C, G). Larval post-winter growth rate was faster in larvae that had experienced a food shortage before winter (food, $\chi^2 = 11.42$, df = 1, $P < 0.001$; Fig. 2D, H), and this to the same extent across species (species × food, $\chi^2 = 2.93$, df = 3, $P = 0.402$) and across photoperiods (food × photoperiod, $\chi^2 = 0.37$, df = 1, $P = 0.543$; species × food × photoperiod, $\chi^2 = 0.93$, df = 3, $P = 0.819$; Fig. 2D, H).

Seasonal time constraints and pre-winter food shortage on adult dry body mass

Overall, dry mass was slightly higher under late than early photoperiod (photoperiod, $\chi^2 = 21.52$, df = 1, $P < 0.001$; Fig. 3A, D) and was highest in *C. ornatum* followed by *C. hastulatum* and *C. mercuriale*, with *C. puella* expressing the lowest dry mass (species, $\chi^2 = 17.66$, df = 3, $P < 0.001$; Fig. 3A, D). However, species differed in their final dry mass in response to changes in photoperiod (species × photoperiod, $\chi^2 = 46.85$, df = 3, $P < 0.001$). Whereas individuals of *C. ornatum* emerged heavier under late than early photoperiod, we found the opposite pattern in *C. puella* (Fig. 3A, D) and did not detect any differences comparing early and late photoperiod in *C. hastulatum* and *C. mercuriale* (Fig. 3A, D).

Dry mass was not affected in any treatment combination by a pre-winter shortage of food (all $P > 0.15$; Fig. 3A).

Seasonal time constraints and pre-winter food shortage effects on adult physiology

Mass-corrected fat content was biggest in *C. mercuriale*, followed by *C. hastulatum* and *C. puella*, and was lowest in *C. ornatum* (species, $\chi^2 = 18.25$, df = 3, $P < 0.001$; Fig. 3B, E). Fat mass was lower in the late than in the early photoperiod (photoperiod, $\chi^2 = 8.75$, df = 1, $P = 0.003$), however, this pattern entirely stemmed from *C. puella*, and other species did not differ between photoperiodic treatments (species × photoperiod, $\chi^2 = 23.66$, df = 3, $P < 0.001$; Fig. 3B, E). Fat mass was lower in animals that experienced the pre-winter food shortage (food, $\chi^2 = 10.14$, df = 1, $P = 0.001$), however, species differed in their response (species × food, $\chi^2 = 10.22$, df = 3, $P = 0.017$), with only *C. puella* showing a tendency for lower fat mass under low than high pre-winter food supply, but *C. mercuriale* showing a tendency for the reverse pattern (Fig. 3B, E). Further, the effect of photoperiod was greater under high food supply than under low food supply (photoperiod × food, $\chi^2 = 6.98$, df = 1, $P = 0.008$; Fig. 3B, E). No other significant effects were detected (all $P > 0.160$).

Mass-corrected phenoloxidase activity was higher in *C. hastulatum* compared to the other species (species, $\chi^2 = 44.24$, df = 3, $P < 0.001$; Fig. 3C, F). The effect of the photoperiod treatment differed among species (photoperiod × species, $\chi^2 = 8.45$, df = 3, $P = 0.038$; Fig. 3C, F): while phenoloxidase activity did not differ between photoperiods in *C. ornatum*, *C. mercuriale*, and C. *hastulatum*, *C. puella* expressed a higher phenoloxidase activity in the late than in the early photoperiod (Fig. 3C, F). Pre-winter food shortage did not affect phenoloxidase activity (food, $\chi^2 = 0.05$, df = 1, $P = 0.816$), nor did species differ in their response to food supply (species × food, $\chi^2 = 1.97$, df = 3, $P = 0.578$; Fig. 3C, F). No other significant effect was detected (all $P > 0.397$).

Discussion

Effects of seasonal time constraints on development rate

All species accelerated development under time constraints. Faster development under seasonal time constraints is a common strategy to avoid too-strong delays in the date of transition (reviews in Dmitriew [2011] and Stoks and Córdoba-Aguilar [2012]). Late transitions toward the adult stage result in worse conditions for survival (Altwegg and Reyer 2003), a lower lifetime mating success (Anholt et al. 1991, De Block and Stoks 2005), and a lower number and quality of offspring (Johansson and Rowe 1999), for example due to inferior oviposition sites.

There was some support for the hypothesis for less compensation in development rate under time constraints in species with a longer reproductive season. At the phenotypic level, this was upheld when comparing the species with the longest adult reproductive season, *C. puella*, against the three other species. Only *C. puella* had an emergence date about two weeks later under the late photoperiod, despite having compensated about six weeks of the simulated two-month delay at hatching under the late photoperiod. Our data are well supported by a field study of Lowe et al. (2009), with late-hatched individuals of *C. puella* developing more than five weeks faster than early-hatched ones. We expected the greater delay in the species with the longest reproductive season because those face less strong selection on early transition to avoid missing out on reproduction. However, even though reproductive season differs by up to ~1.5 months comparing *C. ornatum*, *C. hastulatum*, and *C. mercuriale*, these species managed to keep their date of transition nearly constant and fully compensated for the two-month delay. Some species with fairly long reproductive seasons may not delay development even if hatched late because there may still be fitness costs to late emergence. Indeed, there is some evidence that late emergence can decrease fitness in odonates (Johansson and Rowe 1999, De Block and Stoks 2005), however, in, e.g., *C. mercuriale*, day of emergence does not affect individual mating success (Purse and Thompson 2005). The phylogenetic analysis provided further support for the hypothesis of a greater increase in development rate under time constraints in species with a shorter reproductive season, as it suggested a negative covariation between time-constraint-induced plasticity in development rate and the length of the reproductive season.

Effects of pre-winter food shortage on development rate and growth rate

In contrast to our prediction, the delay of emergence after pre-winter food shortage did not positively covary with the length of the reproductive season but was similar in all four species. Further, the increase in growth rate after food deprivation did not negatively covary with the reproductive season length but was similar in all four species. As in anurans (Dahl et al. 2012) and another *Coenagrion* species (Dmitriew and Rowe 2005), all four species fully compensated for the lower mass accumulated under the pre-winter food shortage by increasing post-winter growth rates and to a lesser extent by delaying emergence. Increases in growth rate after a starvation period to compensate for mass losses are widespread (reviewed in Metcalfe and Monaghan [2001] and in Dmitriew [2011]) and mass compensation at transition may be full (Stoks et al. 2006*b*, Dahl et al. 2012) or incomplete (Dmitriew and Rowe 2005, De Block et al. 2008*b*). In contrast, in *Lestes* damselflies from temporary ponds, the compensatory response to transient food stress was mainly by delaying the age of transition rather than by increasing growth rate (De Block et al. 2008*b*). These differences in response may be driven by the much faster life history of *Lestes* species (larval stage of ~2–3 months compared

to *Coenagrion* species (larval stage of ~8–9 months), precluding a further growth acceleration.

Effects of seasonal time constraints on growth rate and mass at transition

All species accelerated growth under time constraints, as predicted by optimality models and confirmed in several empirical studies (reviewed in Dmitriew 2011). Furthermore, in line with our expectation, we saw a gradual acceleration of growth rate across species that covaried negatively with the length of the reproductive season. This was also confirmed by our phylogenetic analysis indicating higher plasticity in growth rates of species with a shorter reproductive season. Note that the smaller time-stress-induced growth acceleration in *C. puella* does not seem to stem from a physiological limitation, since former studies proved larval *C. puella* to be able to grow at a faster rate under the threat of predation and also under more severe photoperiod time constraints (e.g., Mikolajewski et al. 2013).

Species also differed in the timing of the response in growth rate to larval time constraints, with *C. puella* responding strongest by accelerating growth before winter, *C. ornatum* by growing intermediately before and after winter, and *C. hastulatum* as well as *C. mercuriale* by increasing growth rate strongest after winter. Larvae of *C. puella*, the species with the longest reproductive season, are more likely to hatch in August and September than the other species (Fig. 1). *Coenagrion puella*, therefore, faces a larger threat of not being able to accumulate enough energy reserves to survive winter (De Block et al. 2007). This may explain their relative greater growth acceleration at the late photoperiod before winter.

In line with species differences in growth responses, time constraints generated a reduction in mass only in *C. puella* at transition. Mass is a key fitness trait in odonates (Sokolovska et al. 2000). This variation in mass responses reflects the patterns observed in a wide array of taxa, showing both cases where body mass at transition to time constraints was kept constant (e.g., Nylin et al. 1996, Rolff et al. 2004) and was reduced (e.g., Johansson and Rowe 1999, Stoks et al. 2006*b*, Mikolajewski et al. 2013). Odonates increase their body mass considerably after emergence (Anholt et al. 1991). Timing of transition should strongly depend on the growth/mortality rate ratio in alternate habitats, which might differ among species (Werner 1986). The smaller body mass under time constraints in *C. puella* might not be maladaptive, as it may reflect an adaptive shift in the balancing of growth-related mortality rates across metamorphosis. Given that *C. puella* has the longest reproductive season, hence more time to compensate mass in the adult stage, it may forego the costs of a complete mass compensation within the larval stage (see also Strobbe and Stoks [2004]). This and the constant intermediate growth rate acceleration before and after winter also might explain the increase body mass at emergence in *C. ornatum* under late photoperiod. Because of its extremely brief reproductive season, *C. ornatum* lacks time to compensate larval mass deficits during the larval phase in the adult phase and rather accelerates growth during larval development.

Effects of seasonal time constraints and pre-winter food shortage on adult physiology

Even though costs of rapid growth are widespread and essential to understanding why not all animals grow maximally (reviewed in Dmitriew [2011]), we detected no consistent evidence for short-term costs provoked by rapid growth rates. We did find the expected reduction in fat storage, yet only in *C. puella*, the species with the longest reproductive season. Fat represents the most important energy reserve storage in insects (Wigglesworth 1965) and is linked to mating success (Plaistow and Siva-Jothy 1996). A reduced fat storage in fast-growing animals has been shown before (Stoks et al. 2006*b*, De Block et al. 2008*b*), but is not always detected (e.g., Rolff et al. 2004). *Coenagrion puella* may not have been able to keep fat content constant under time constraints, given that this species showed a costly up-regulation of PO activity (De Block and Stoks 2008). Consistent with this, fat content was not reduced after compensatory growth associated with pre-winter food shortage, as PO activity was not up-regulated. In no species was more rapid growth associated with a reduced PO activity, and in *C. puella*, more rapidly growing animals under time constraints even expressed a higher PO activity. The observed increased investment in immune function by *C. puella* may go together with the hypothesized strategy of compensating after transition, since phenoloxidase, among others, is involved in wound repair (Siva-Jothy et al. 2005), with early-life repair efficiency promoting enhanced survival and life span (Cichon 1997). Previous studies have documented a lowered investment in immune function when growth was accelerated (Rolff et al. 2004, Stoks et al. 2006*b*), but again, this response is not general (e.g., Dmitriew et al. 2007). This inconsistency among studies is not surprising, as trade-offs may be multivariate (Travis 1989, Mikolajewski et al. 2013) and increased energy allocation toward growth may be at the cost of a multitude of other functions. Moreover, costs of rapid growth may only become apparent under stressful conditions late in adult life (reviewed in Dmitriew [2011]). Also, increases in food intake may offset physiological trade-offs (van Noordwijk and de Jong 1986), yet likely will result in ecological costs such as an increased risk of predation (Anholt and Werner 1995, Stoks et al. 2005). Finally, fast-growing species may have evolved buffering physiological mechanisms over time to compensate and reduce the costs of enhanced growth, and therefore these costs may not be so marked anymore.

Conclusions and perspectives

While species differ widely in their response to seasonal time constraints in the larval stage, few

empirical studies have tried to unravel differences between closely related species, and all these focused strictly on selective factors associated with the stability of the larval habitat (e.g., Morey and Reznick 2004, Gomez-Mestre and Buchholz 2006, De Block et al. 2008*a*). We here extended these efforts by documenting that time constraints associated with the length of the adult reproductive season may predictably contribute to species differences in their response to time constraints imposed in the larval stage. Given that species often differ strongly in the length of the period suitable for reproduction (Verberk et al. 2008), we hypothesize that this may frequently and predictably shape species-specific life-history plasticity to time constraints imposed in the larval stage.

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Supplemental Material

Ecological Archives

The Appendix is available online: http://dx.doi.org/10.1890/14-0262.1.sm